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U. S. DEPARTMENT OF AGRICULTURE

BUREAU OF ANIMAL INDUSTRY

JOHN R. MOHLER, CHIEF

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# THE OPHTHALMIC AND INTRADERMIC TESTS FOR GLANDERS



WASHINGTON  
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1919



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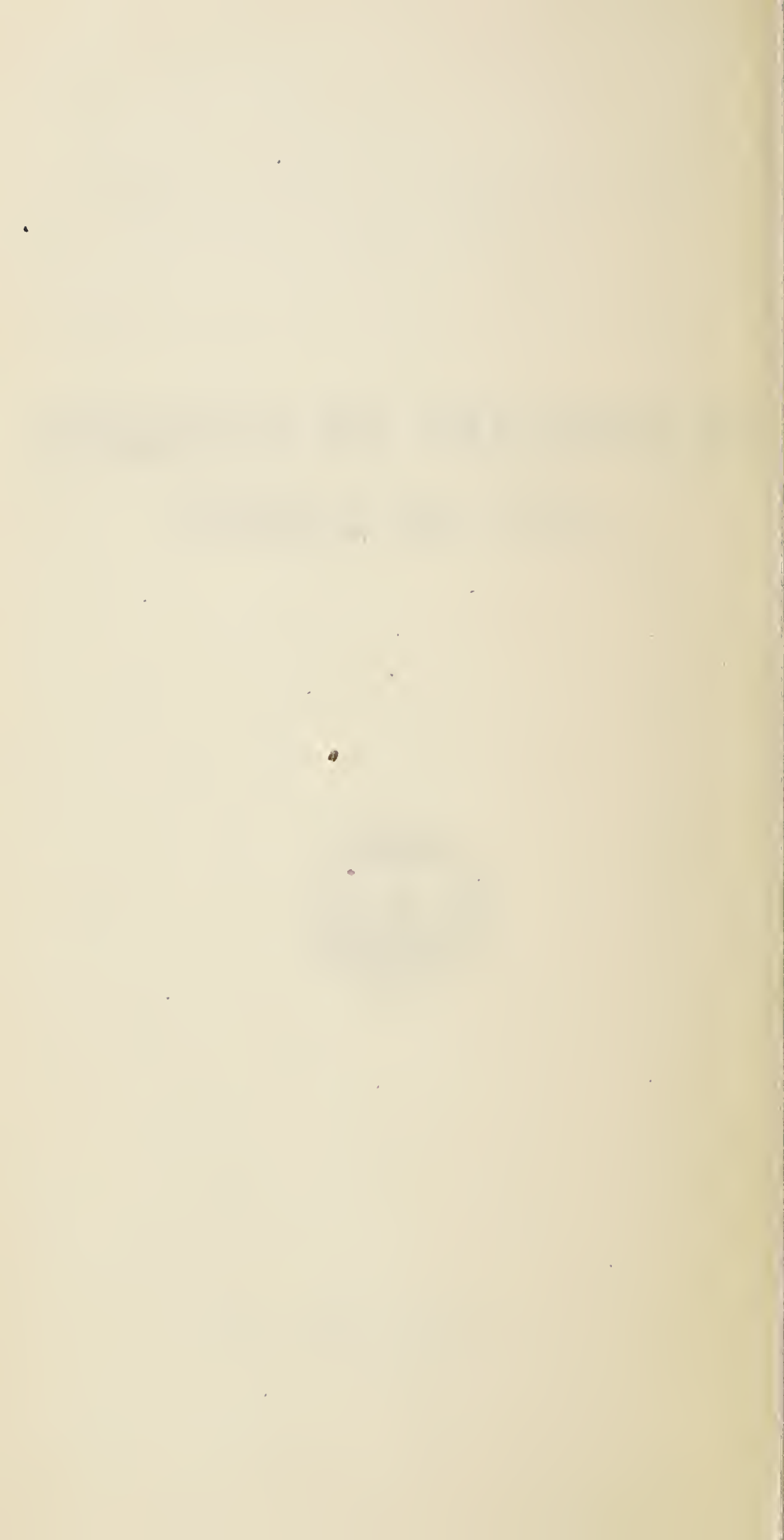
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# UNITED STATES DEPARTMENT OF AGRICULTURE.

## BUREAU OF ANIMAL INDUSTRY.

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### THE OPHTHALMIC AND INTRADERMIC TESTS FOR GLANDERS.

In the United States the subcutaneous test, to all intents and purposes, has ceased to be used as a practical method for the field diagnosis of glanders, having been supplanted by the ophthalmic test. Experience gained through four years' use of the ophthalmic test has shown that in many ways it is superior to the subcutaneous test as a practical method of diagnosis.

Since the beginning of the European war another method of applying mallein has been used extensively on Army horses in Europe. This is known as the intradermo-palpebral, or intradermic, test. In 1914 Lanfranchi recommended the subcutaneous injection of mallein in the lower lid, and later in the same year Drouin and Naudinat described the intradermic injection of mallein, and formulated principles which should be observed in interpreting it. Other observers have reported upon this method and it has been adopted and used by the French, British, and Italian armies with great satisfaction. Extensive tests carried out by the Veterinary Corps of the United States Army, as well as by the Bureau of Animal Industry, have likewise indicated that the intradermic test is a reliable and practical method for diagnosing glanders.



## TWO METHODS RECOGNIZED FOR OFFICIAL TESTING.

In view of these facts the bureau has decided to recognize the intradermic method as a means for detecting glanders in horses and mules offered for interstate shipment. At the present time, therefore, two methods are recognized for official testing—(1) the ophthalmic test; and (2) the intradermic test.

On the whole the intradermic test is regarded as somewhat more delicate and reliable than the ophthalmic test, provided it is applied by a skilled operator.

The ophthalmic test is very simple in application and under favorable conditions the results are excellent. It has the disadvantage, however, that the most prominent evidence of a reaction, viz, a discharge from the eye, may be obliterated accidentally by the horse in rubbing the eye or purposely by an unscrupulous attendant.

The intradermic test has the advantage of producing a reaction (swelling of the lids) that can not be obliterated; but on the other hand it has the disadvantage of a considerably more difficult technique.

The following description of the methods of application and of the principles to be observed in judging the results of these two tests are offered for the information of veterinarians.

## THE OPHTHALMIC METHOD.

## TECHNIQUE.

**Equipment.**—The only equipment needed for the application of the ophthalmic test is a small, soft, clean camel's-hair brush or a glass medicine dropper with a rounded end.

**Mallein to be used.**—The Bureau of Animal Industry will furnish to officials for official use the concentrated crude mallein which is used without dilution for making the test. Diluted mallein such as is used in subcutaneous tests is unsuitable for the ophthalmic test. Strong solutions of precipitated mallein may be employed, but are not furnished by the Bureau of Animal Industry.



**Method of application.**—The mallein is to be applied only when there is no evidence of conjunctivitis or other inflammatory condition of the eye. In the presence of such conditions the test should not be applied.

It is best, as a routine procedure in order to avoid mistakes and to simplify record keeping, to apply the test uniformly to the right eye, the left eye being untreated. When an exception is made to this rule, suitable note of the fact should be taken.

The head of the animal to be tested should be held firmly by an assistant and two or three drops of the concentrated ophthalmic mallein should be placed in the lower conjunctival sac by means of the medicine dropper, or the mallein may be applied by means of the camel's-hair brush which is first dipped into the mallein and then brushed lightly over the conjunctiva of both the upper and lower lids.

#### THE REACTION AND JUDGMENT OF RESULTS.

Increased lachrymation, some reddening of the conjunctiva, and a slight photophobia in the eyes of healthy animals are usually observed. No significance should be attached to these symptoms. They appear very soon after the introduction of the mallein and disappear within a few hours. The characteristic reaction in the case of glandered animals appears as a rule not earlier than the fifth or sixth hour and increases from that time until the twelfth or eighteenth hour, when it may begin to subside. It is usual for the reaction to last for from 24 to 36 hours and at times longer.

The characteristic reaction consists in a distinct, purulent discharge from the tested eye while the untested eye remains unaffected. The conjunctiva of the lids and the eyeball should always be included in the examination after an inspection of the discharge. By comparison with the untested eye it may be determined whether there is inflammation of the conjunctiva. The eyelids should be examined for indications of swelling. The indications of a reaction, it should be remembered, may be wholly or

partly obliterated (either intentionally by attendants or accidentally by the animals). In such cases dry pus may be found adhering to the hair around the eye and at times freshly formed pus may be observed in the conjunctival sac when the lid is retracted.



FIG. 1.—An average reaction to the ophthalmic test.

In the absence of any secretion from the eye the test should be regarded as negative. When there is a clear mucous discharge or continued lachrymation persisting to the twelfth or sixteenth hour after application of the mal-  
lein the reaction is to be regarded as doubtful and the test

may be repeated immediately, using the same eye, when as a rule the results are more sharply defined.

The ophthalmic test should not be made more than three times on the same animal within a short period. Experiments show that after the third application within three months the reaction usually loses its intensity in positive cases and in subsequent tests may fail entirely. When the results of the second test in doubtful cases are not clearly decisive, blood should be drawn and submitted to a laboratory for the complement-fixation test. Animals may be retested at intervals of six months with satisfactory results.

The results of the test should be recorded as follows:

N=Negative. Eye unchanged.

S=Suspicious. Seromucous discharge.

P+=Positive. Seromucous discharge, with purulent flakes.

P++=Positive. Distinct, purulent discharge.

P+++ =Positive. Purulent discharge, with swelling of the eyelids.

P++++ =Positive. Strong purulent discharge, with swelling and gluing together of both lids.

## THE INTRADERMIC METHOD.

### TECHNIQUE.

**Equipment**—A suitable syringe and needle are essential to the proper performance of this test. A 1-cubic centimeter syringe, graduated in tenths and provided with a set screw which regulates the quantity to be injected, is very desirable. The syringe should be provided with several fine short needles from 10 to 15 millimeters long.

**Mallein to be used.**—The experience of the French authorities seems to show that mallein prepared by diluting one part of the concentrated crude mallein with three parts of a 0.5 per cent solution of carbolic acid in water is satisfactory. Other dilutions or modes of preparation should not be used until extensive trial has proved that they are as effective as the preparation described above.



The Bureau of Animal Industry is prepared to furnish this diluted mallein for official intradermic tests upon request of officials of State, county, or municipal governments.

**Method of application.**—The mallein is applied only when there is no inflammatory condition of the eye of the

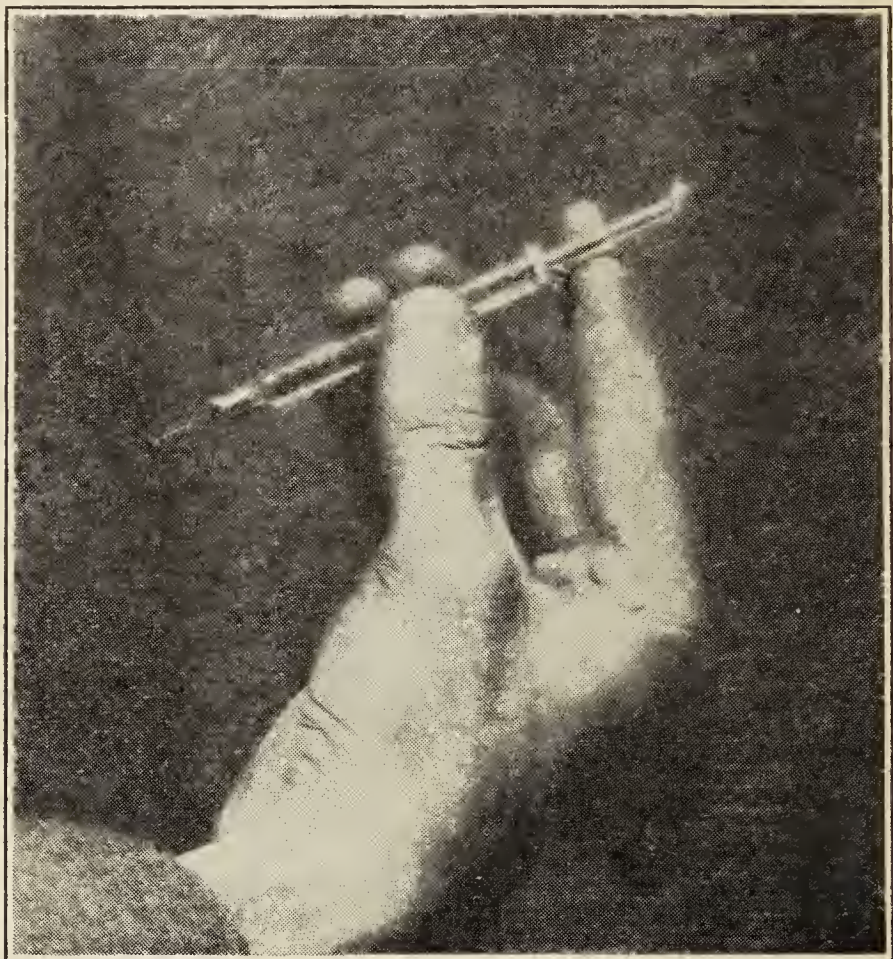


FIG. 2—Inserting the needle. (Courtesy of Lieut. Col. Marshall, U. S. A.)

animal to be tested. Unless conditions in individual cases prevent, the right eye should be selected for the injection. If for any reason the left eye is used, that fact should be noted in the records. The services of an assistant are necessary to facilitate the work of the operator. A twitch should be applied to the upper lip or to the ear so that the head of the animal may be held perfectly still



during the operation. A staff of two or three assistants facilitates the work of the operator when many tests are to be made.

The injection is made into the dermis of the lower lid about one-third of an inch from the edge and midway between the inner and outer canthi. The point of injec-



FIG. 3.—Injecting the mallein. (Courtesy of Lieut. Col. Marshall, U. S. A.)

tion may be disinfected if desired, but in practice it has been found to be unnecessary. In the case of lids which are dirty or greasy, the site of injection should be wiped off with a cotton tampon wet with alcohol or ether.

The dose of mallein to be injected should be one-tenth of a cubic centimeter. After the syringe is completely filled, the needle attached, and the set screw so fixed as to



permit the injection of one-tenth of a cubic centimeter, the syringe is grasped between the thumb and middle finger of the right hand while the index finger operates the stem of the plunger. The back of the right hand rests upon the head of the horse so as to follow any unexpected movement. The thumb and index finger of the left hand grasp the skin of the lid so as to make a horizontal fold of the lower



FIG. 4.—Method of inserting the needle: animal restrained with twitch. (Courtesy of Lieut. Col. Marshall, U. S. A.)

lid into which the needle is introduced for a distance of about one-eighth of an inch and as near as possible to the surface of the skin and parallel with it. *It is important to remember that the injection should be made into the skin and not beneath it into the subcutaneous tissues.* When the injection is well made, it produces a small, distinctly outlined swelling due to the stretching of the tissues of the skin by the injected fluid.

Instead of making a fold in the skin of the eyelid with the fingers of the left hand, the left thumb may be placed just below the eye with the fingers resting over the eye. (See figs. 4 and 5.) The skin of the lower lid is drawn tight by the thumb and the injection made as previously described. When the proper instruments are provided and when the animal is properly immobilized, the technique is not very difficult and a little practice will render any practitioner proficient.



FIG. 5.—Method of injecting mallein without twitch. (Courtesy of Lieut. Col. Marshall, U. S. A.)

#### THE REACTION AND JUDGMENT OF RESULTS.

With the majority of horses a limited edema of the lower lid appears in the first two or three hours following the injection. This swelling, which results from the irritation caused by the injected mallein, is not particularly sensitive and persists for a comparatively short time. If the injection is made in the morning the irritative swelling



usually disappears during the following night or subsides noticeably on the following day. No significance should be attached to it. In healthy animals the eye itself remains normal. In animals affected with glanders the specific reaction becomes marked after about 10 or 12 hours and attains its maximum usually from the twenty-fifth to the thirty-sixth hour. It usually lasts two or three days and finally subsides without leaving any trace except for a certain local thickening of the connective tissues.

In the typical reaction there is an extensive edema, hot and extremely sensitive, involving not only the lower lid but also all the tissues surrounding the eye, including the upper lid, so that the eye is almost closed. The horse flinches at the slightest attempt to touch the parts. The conjunctiva is markedly congested, and from the inner angle of the eye there flows a more or less abundant mucopurulent secretion, which collects as a yellowish deposit on the eye.

The reaction described above is typical and pronounced. There are cases, however, in which the reaction is localized in the lower lid. The conjunctivitis and the exudate are as marked as in the complete reaction, but the edema, though voluminous, is circumscribed in the lower lid and in the tissues of the angular space above the zygoma. The swelling in cases of this kind exhibits the same characters as in the more extensive reaction, being hot and sensitive, and it persists in the same way as in the more complete reaction. It may even extend below the zygoma, and is generally accompanied with fine, sinuous lines marking the course of the lymphatics toward the sublingual gland, which on palpation is found to be sensitive. Although this reaction involves the lower lid only, it is as easy to determine as in the reaction which involves both lids.

Between the reactions which have just been described as positive indications of glanders and the slight edema which develops soon after the injection in healthy horses there are some intermediate grades which must be carefully considered. In such cases conjunctivitis may be absent or very slight. The edema affects only the lower

lid and is diffuse, or it may be circumscribed about the point of injection in the form of a crescent. In doubtful cases the animal should be tested again after five or six days, using the left eye and putting the intradermal injection into both the upper and lower lids, or a sample of the blood may be taken and submitted to a laboratory for the complement-fixation test.

In the case of subjects which have been previously tested, it is usually found that there is some thickening of the connective tissue about the eye which was used for the test, and the edema following the second test on the same eye is diffuse and the conjunctivitis is slight. When the upper lid is used there is a swelling which fills up the orbital fossa and there is a falling of the lid.

The results of the test should be recorded as follows:

(The letter E is used to indicate the edema and the figures 1 and 2 to indicate the lower and upper lids, respectively. The letter P is used to indicate a discharge from the eye with + signs to denote the degree.)

N = Negative. Eye unchanged.

S = Suspicious or doubtful. Slight diffuse swelling of lower lid. Little or no discharge.

E 1 = Positive. Characteristic edema of lower lid.

E 1 and 2 = Positive. Characteristic edema of both lower and upper lids.

E 1 P + = Positive. Edema of lower lid and seromucous discharge with purulent flakes.

E 1 and 2 P ++ = Positive. Edema of both lids; distinct purulent discharge.

No mallein test has been devised that will detect all glandered animals. Careful physical examinations always should be made, and no animal should be released if it exhibits clinical symptoms of glanders, even though it gives no reaction to mallein. In such cases recourse should be had to a blood test if the clinical manifestations do not warrant a diagnosis.











